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13. ABSTRACT (Maximum 200 words)  Biological effects of low frequency underwater sound are most pronounced in and near tissues that contain gas, such as the lung. The response of gas bodies <i>in vivo</i> to low frequency underwater sound was characterized through a series of experimental and theoretical investigations. This report summarizes work done for the first stage of the project and, therefore, concentrates on the response of lung to underwater sound. A specially designed acoustic exposure system, capable of generating maximum acoustic fields of ~200 dB re 1 $\mu$ Pa over the 100-2500 Hz range, was tested and implemented for these investigations. The system was employed in three modes of operation (traveling wave, pure pressure, and pure velocity) in experiments designed to test specific hypotheses regarding the mechanism for sound-induced lung damage. An acoustic scattering technique was employed to characterize the response of gas bodies to sound exposure. Through a series of experiments, we determined that acoustic pressure is the key parameter for characterizing the threshold for lung damage produced by low frequency underwater sound. Results of these investigations will be directly applicable to the next stage of the project, which focuses on the response of intestinal gas to exposure to low frequency underwater sound.				
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## Final Reporting for Office of Naval Research Award No. N00014-99-1-1053

### Response of Biological Tissues to Low Frequency Underwater Sound

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#### I. INTRODUCTION.

The biological effects of low frequency underwater sound are most pronounced in and near tissues that contain resonant gas bodies. Murine lung provides an excellent illustration of the response of gas bodies *in vivo* to low frequency sound. Lung responds to low frequency sound as a resonant body that can be modeled using linear theory. The guiding hypothesis of this project is that the response of murine lung to low frequency sound is representative and predictive of the response of other gas bodies *in vivo*. Through a series of experimental and theoretical investigations, we characterized the response of gas bodies *in vivo* to low frequency sound and worked toward identifying the mechanisms for tissue damage in and near resonant gas bodies *in vivo*. Thresholds for damage to gas-containing tissues, such as the lung and intestine, were determined over the 500-2500 Hz frequency range.

There are three main goals associated with this project:

1. Characterize the response of resonant gas bodies to exposure to low frequency underwater sound.
2. Determine the thresholds for murine lung hemorrhage using traveling wave, pure pressure and pure velocity fields. Investigate the thresholds for murine lung hemorrhage at frequencies of 500-2500 Hz.
3. Investigate damage to the intestine from exposure to low frequency underwater sound.

This report summarizes work done for the first stage of this project and, therefore, is limited to investigations of the lung.

#### II. EXPOSURE SYSTEMS.

Two exposure systems were used to generate low frequency underwater sound in the laboratory. One system was the G40 calibrator obtained from the U.S. Navy Underwater Sound Reference Detachment. The G40 calibrator is an open, inertial impedance calibration system that provides a convenient method for exposing small laboratory animals and, at the same time, provides a high level of accuracy in specification of exposure pressure. This system is most useful for frequencies below ~1000 Hz, however, it was particularly useful for testing specific hypotheses over the complete 100-2500 Hz frequency range.

The second system used to generate acoustic fields for this project was a specially designed traveling wave tube system (i.e., ratabrator). This system is capable of generating maximum acoustic amplitudes on the order of 200 dB re 1  $\mu$ Pa over the 500-2500 Hz frequency range. The system can be driven in three modes of operation. The system can be used to generate a traveling wave field in the exposure chamber. In this mode of operation, the pressure is linearly proportional to the particle velocity and the constant of proportionality is the acoustic impedance of the propagating medium. The system can also generate a "pure pressure" field. In this mode of operation, the acoustic pressure is maximized and the particle velocity is minimized. Last, the

system can generate a “pure velocity” field where the particle velocity is maximized and the pressure is minimized. The ability to generate these three different types of acoustic fields was necessary in order to test specific hypotheses regarding acoustic mechanisms for effects of low frequency sound on biological tissues.

Figure 1 illustrates field measurements made in the three modes of operation of the traveling wave tube system. Figure 1a are results for the traveling wave mode of operation, Figure 1b are results for “pure pressure” mode, and Figure 1c are results for “pure velocity” mode. Measurements were performed in the chamber filled with degassed, deionized water at room temperature. Measurements were performed with a hydrophone (B&K Model 8103) and neutrally buoyant accelerometer. In each graph, the x-axis is exposure frequency (Hz) and the y-axis is pressure (for hydrophone measurements) or pressure multiplied by acoustic impedance of water (for accelerometer measurements). Note that for the traveling wave case, the measurements support the plane wave relationship between pressure and particle velocity.

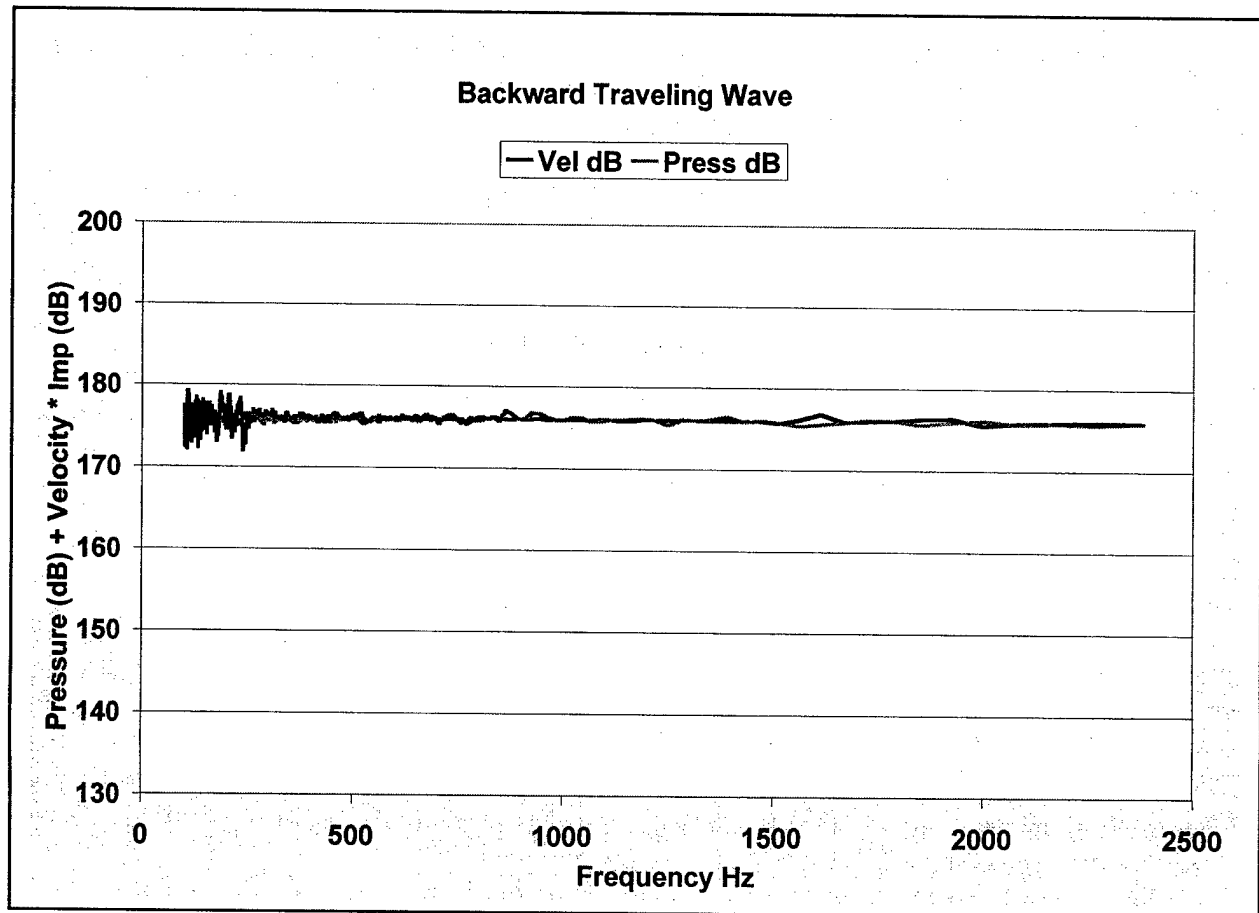


Figure 1a. Traveling wave mode of operation. Note pressure is equal to the product of impedance and velocity.

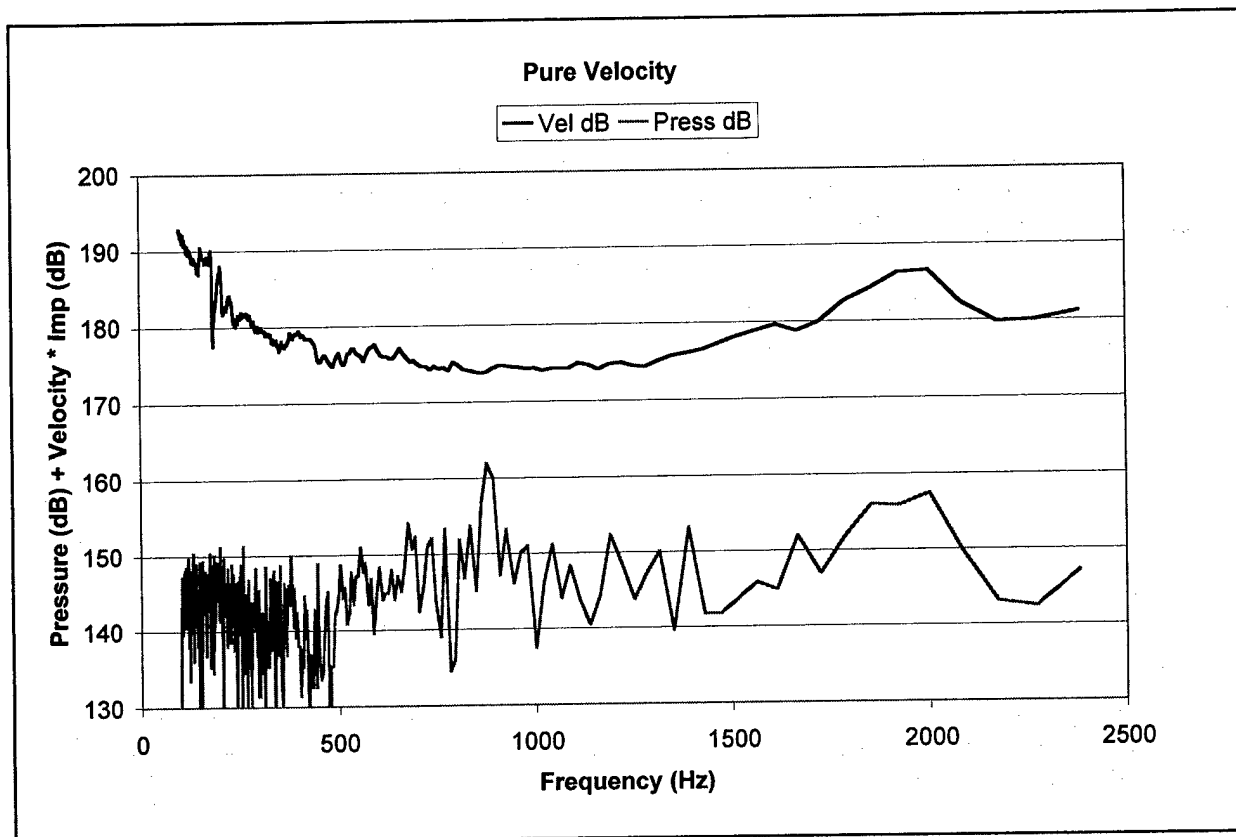


Figure 1b. Pure velocity mode of operation. Note velocity is maximized and pressure is minimized.

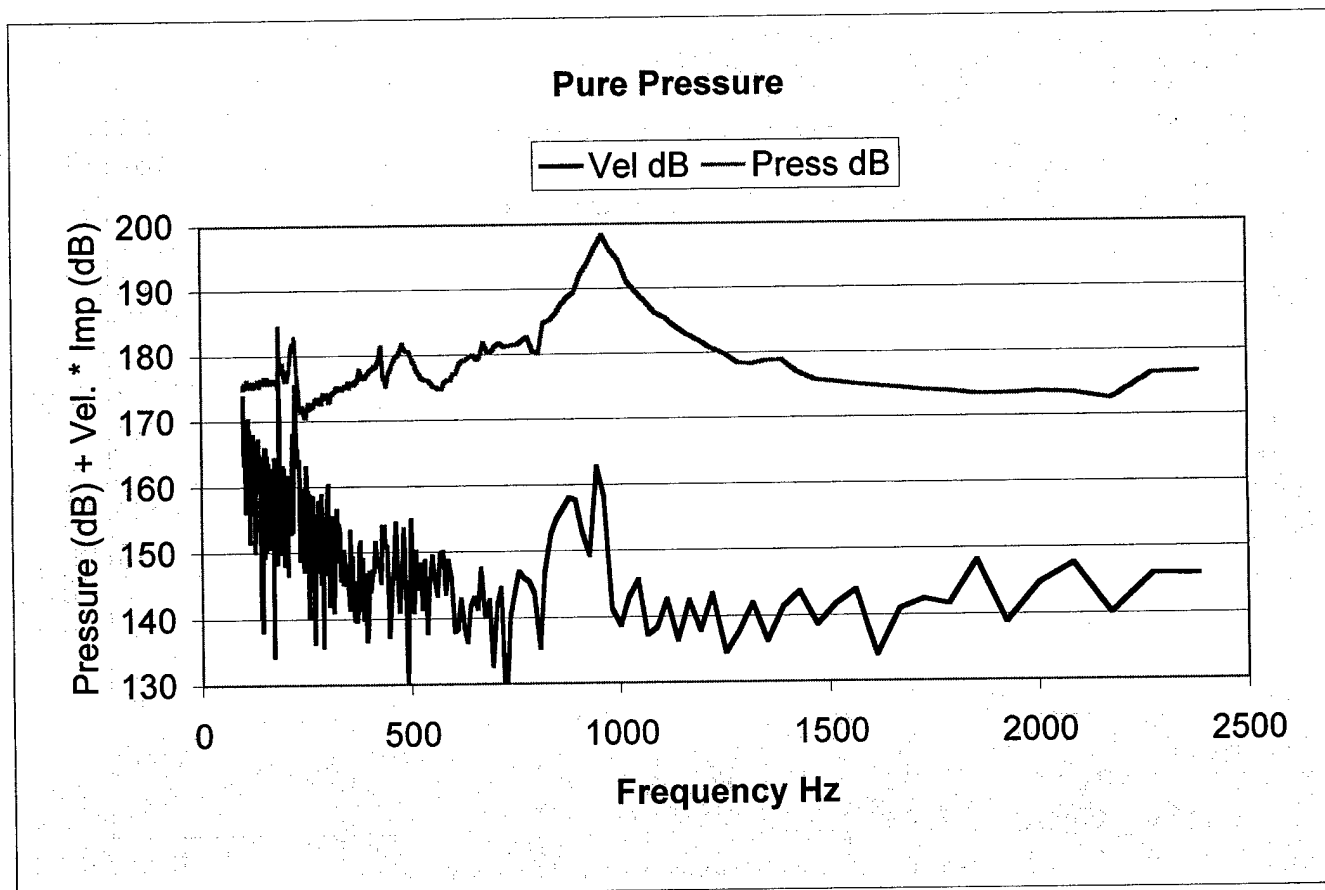


Figure 1c. Pure pressure mode of operation. Note pressure is maximized and velocity is minimized.

### III. LUNG STUDIES.

Investigations in an earlier project in our lab determined that the air-filled lung is susceptible to damage from exposure to low frequency underwater sound. Our guiding hypothesis for this project is that the characteristics of the response of lung to low frequency sound are representative of other gas bodies in vivo. Efforts in the first phase of this project, concentrated on characterizing the response of resonant gas bodies and on obtaining further information to elucidate the mechanism for sound-induced lung damage. The text below briefly summarizes two series of investigations of the effects of low frequency sound on the lung.

*Lung Resonance.* Mice (CD1) were anesthetized with IM injections of rompun (10 mg/kg) and ketamin (200 mg/kg) and torsos were shaved and depilated to minimize trapped air on the skin surface. A 25 gauge catheter was implanted surgically into the trachea and tied securely in place with suture. The animal was prepared for underwater ventilation by connecting the catheter via flexible tubing to a small animal ventilator. The animal was then placed on a small animal holder and placed in the exposure chamber of the traveling wave tube exposure apparatus. A hydrophone was secured to the small animal holder at distance of ~ 1 cm from the surface of the murine chest.

The acoustic field near the surface of the chest as a function of frequency was measured for a series of mice and rats. The resonance frequency was considered the frequency at which the total acoustic field was maximum. An example of the resonance of murine lung exposed to a traveling wave field is shown in Figure 2 below. Note that at the resonance frequency (~325 Hz) the total acoustic field is maximized. Also, at frequencies above resonance, the presence of the lung decreases the total acoustic field near the lung. The response of the lung to the traveling wave field exposure is the same as that observed in an earlier project using the G40 calibrator. This technique was then used to find the resonance frequency of mice for all subsequent studies.

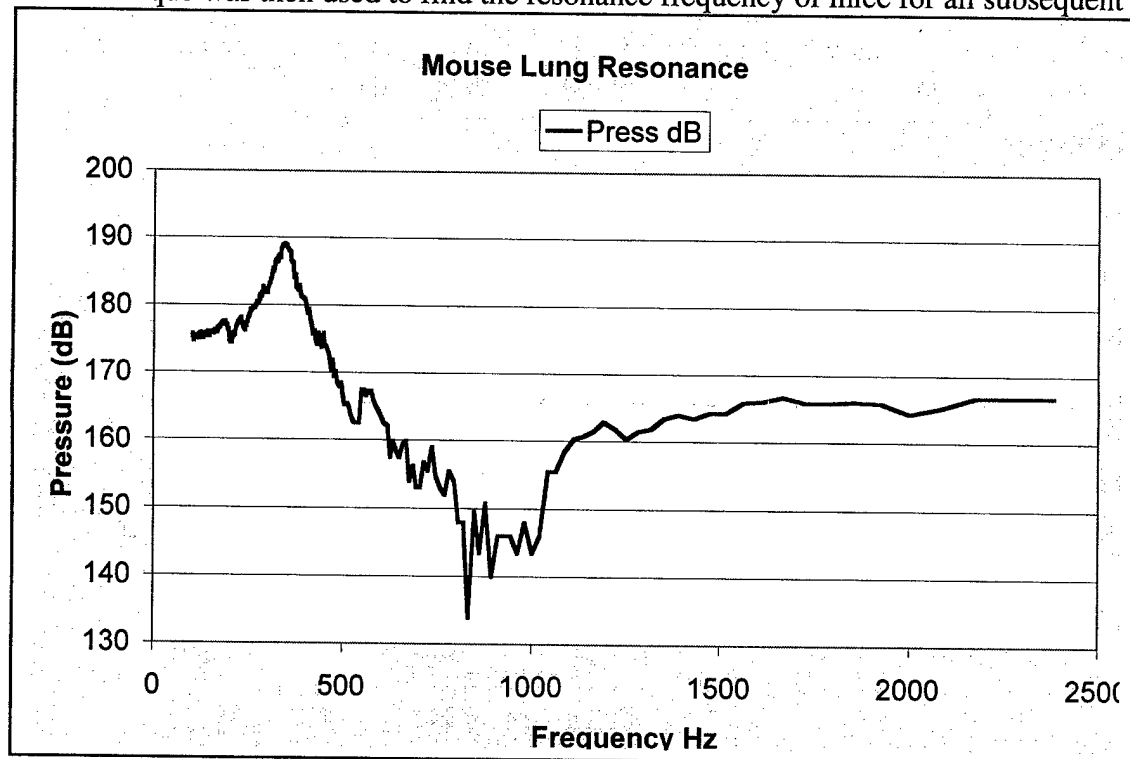


Figure 2. Example of measurements of lung resonance of mouse.

*Thresholds for Lung Hemorrhage.* Previous studies in our lab have indicated that the lung is particularly sensitive to damage from exposure to low frequency underwater sound. The mechanism for this effect of sound on the lung is not completely understood. To aid our understanding of acoustic mechanisms and to identify the appropriate acoustic parameter to characterize the threshold for damage it was necessary to determine the relative importance of the role of acoustic pressure versus acoustic particle velocity in the production of lung damage. The specific objective of this series of experiments was to determine the threshold for murine lung hemorrhage using traveling wave, pure pressure and pure velocity fields. We hypothesized that acoustic pressure is the relevant parameter for defining thresholds for lung damage. As a result, we expected the thresholds for lung damage from exposure to the traveling wave, pure pressure and G40 fields would be comparable.

*Traveling Wave Exposures.* Adult mice (CD1) were prepared for exposure as described above. The exposure system was operated in the traveling wave mode and complete calibrations were performed at the start of every day. For each animal, the resonance frequency of the lung was first determined using the acoustic scattering technique. All exposures were then performed at the lung resonance frequency. Each animal was exposed for 5 minutes, continuous wave. There were five exposure amplitudes (including sham) and six animals per exposure amplitude.

Lung hemorrhage was observed to in mice exposed to the traveling wave field. The hemorrhage appeared as areas of red damage and was qualitatively similar to that observed in our earlier project using the G40 exposure system. The extent of lung damage increased with exposure amplitude. At higher exposure amplitudes, damage was also observed on the surface of the liver near the interface with the lung. In some instance, severe damage to the lung occurred such that air was observed in the chest cavity. Results for this study are summarized in Table 1. For each exposure amplitude, the table reports the mean resonance frequency of the lung, percentage of exposed animals with lung damage, liver damage and air in the chest, and the mean area of lung and liver damage.

Amp. (dB)	Mean $f_{res}$ (Hz)	% Lung	Area Lung (mm <sup>2</sup> )	% Liver	Area Liver (mm <sup>2</sup> )	% Air Chest
193	384 [15.3]	100	60.2 [9.1]	50	9.0 [5.9]	16.7
190	383 [16.3]	50	15.9 [12.8]	0	0	16.7
187	380 [8.0]	33.3	1.0 [0.72]	16.7	3.0 [3.0]	0
183	380 [13.6]	16.7	1.3 [1.3]	16.7	0.08 [0.08]	0
0	—	16.7	0.08 [0.08]	0	0	0

Table 1. Threshold study for traveling wave exposures.

The results obtained with the traveling wave exposure were similar to those obtained in an earlier investigation using the G40 exposure apparatus. For traveling wave exposures, the threshold for lung damage was ~189 dB re 1  $\mu$ Pa. These thresholds are not statistically different from those obtained with the G40 calibrator. Although the thresholds for damage to the lung and liver are the same, the extent of damage was somewhat less for the traveling wave exposure than for the G40 exposure.

*Pure Pressure Exposures.* The experiment detailed above for the traveling wave field was repeated with the pure pressure mode of operation. Animal procedures and exposure protocols were identical to the previous study except animals were exposed to pure pressure fields. Again, 5 exposure amplitudes were investigated with six animals per exposure condition.

Lung and liver hemorrhage and air in the chest were also observed in mice exposed to the pure pressure acoustic fields. Table 2 summarizes the results from this study. These results are essentially the same as those obtained for the traveling wave mode and reported in Table 2. For pure pressure exposures, the threshold for lung damage was ~189 dB re 1  $\mu$ Pa. These thresholds are not statistically different from either those obtained with the traveling wave mode of operation or G40 calibrator. These results are fully consistent with our original hypothesis.

Table 2. Threshold study for pure pressure exposures.

Amp. (dB)	Mean $f_{res}$ (Hz)	% Lung	Area Lung (mm <sup>2</sup> )	% Liver	Area Liver (mm <sup>2</sup> )	% Air Chest
193	360 [9.8]	100	60.2 [13.7]	33.3	6.4 [4.1]	16.7
190	358 [5.9]	83.3	18.6 [3.9]	0	0	0
187	362.7 [6.7]	33.3	0.95 [0.66]	0	0	0
183	360 [3.5]	0	0	0	0	0
0	—	33.3	0.14 [0.08]	0	0	0

*Pure Velocity Exposures.* Recall, our hypothesis for this phase of the project was that acoustic pressure was the appropriate parameter to characterize sound-induced lung damage. Results reported above were consistent with this hypothesis, however, as a final test of this hypothesis, lung hemorrhage was investigated using the pure velocity mode of operation. If our hypothesis is correct we would not expect to see any damage to tissues exposed to the pure velocity fields.

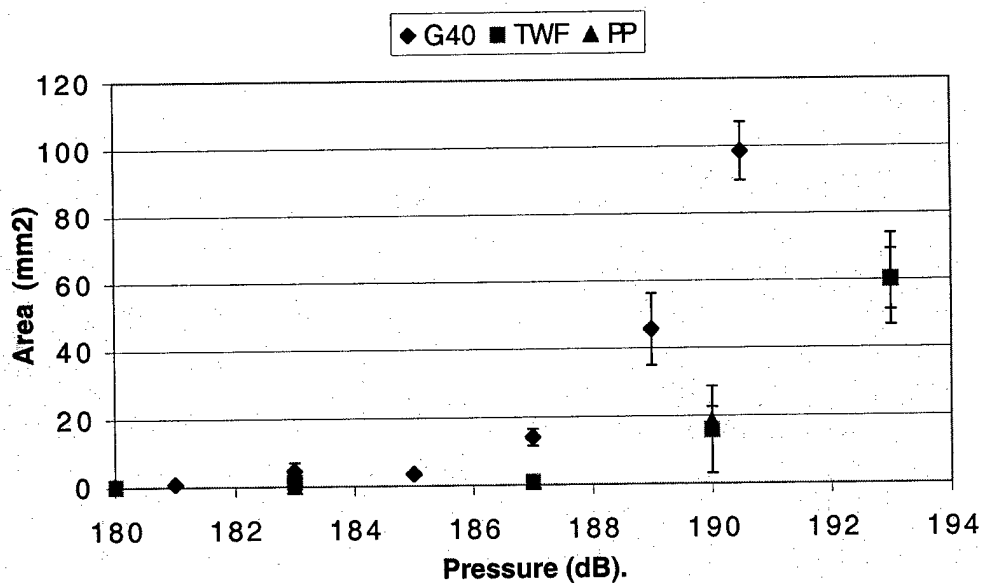
Animal procedures and exposure protocols were as described above except the pure velocity mode of operation was used for exposures. An exploratory study indicated no significant

damage even at amplitudes up to 196 dB re  $1\mu\text{Pa}$ . A primary study was then completed with 6 animals exposed at 196 dB re  $1\mu\text{Pa}$  and 6 animals sham exposed. The results of these studies are shown below in Table 3. Note, no significant damage to the lung or liver was observed for mice exposed to the pure velocity fields.

Amp. (dB)	% Lung	Area Lung (mm <sup>2</sup> )	% Liver	Area Liver (mm <sup>2</sup> )	% Air Chest
196	50	0.1 [0.04]	0	0	0
0	0	0	0	0	0

Table 3. Results of exposures with the pure velocity mode of operation.

A summary of the results of the threshold investigations using the traveling wave, pure pressure and G4 calibrator are shown below in Figure 3. Results of all threshold studies described above are consistent with our original hypotheses. These investigations indicate that acoustic pressure is the relevant parameter to define thresholds for sound-induced lung damage and also that earlier results obtained with the G40 calibrator system are applicable for traveling wave conditions.



#### IV. SUMMARY.



The overall goal of this project is to develop a greater understanding of the response of biological tissues to low frequency underwater sound. Efforts focus particularly on tissues that contain gas (such as the lung and intestine) since these tissues are most susceptible to damage from sound exposure. The first phase of this project (summarized in this report) aimed to develop appropriate exposure systems for use in a laboratory setting, begin investigations of the response of gas bodies to low frequency sound and further characterize the response of lung to exposure to underwater sound. These aims have been accomplished. As described above, we have implemented a specially designed traveling wave tube capable of generating acoustic fields on the order of 200 dB re 1  $\mu$ Pa for three different modes of operation (traveling wave, pure pressure and pure velocity). We have used the acoustic scattering technique to characterize the response of various gas bodies *in vitro* and *in vivo* to low frequency sound exposure. Through a series of threshold studies we determined that acoustic pressure is the key parameter to characterize the effects of low frequency sound on the lung. Completion of these studies and the results of these studies will be directly applicable to the next stages of this project. Remaining efforts will focus on investigations of the response of intestinal gas to exposure to low frequency underwater sound.